

Determination Of Packed Cell Volume by Macro Method

Clinical Importance of PVC

Aim of Presentation

To Know

- What is Packed Cell Volume (PCV)
- Methods for Determination of PCV (Hematocrit)
- Clinical Importance of PCV (Hematocrit)

PCV

- PCV also known as Hematocrit is the volume occupied by Erythrocytes in the given volume of blood and is usually expressed as percentage of the volume of the whole blood sample.

SRI

Hematocrit is also sometimes called packed cell volume (PCV), volume of packed red cells (VPRC), or erythrocyte volume fraction (EVF). The term hematocrit comes from the Ancient Greek words haima (αἷμα, "blood") and krites (κρίτης, "judge"). Together, hematocrit means "to separate blood".

Hematocrit (PCV)

- Hematocrit is derived from Greek words 'Haima' meaning blood 'krites' meaning to Separate. Together Hematocrit means 'to separate blood'

Methods:

1. Macrohematocrit method (Wintrobe Method)
2. Microhematocrit method

Macrohematocrit method (Wintrobe Method)



- **Hematocrit**

- **Males: 44% (38—50%)**
- **Females: 42% (36—45%)**

Apparatus Required:

1. Wintrobe tube
2. Centrifuge machine
3. Pasteur pipette
4. Pricking apparatus to get venous blood sample
5. EDTA vial

PRINCIPLE

- Anticoagulant mixed blood is filled in a tube and centrifuged. Cells settle down towards the bottom because of their greater density leaving the clear plasma on upper side

Wintrobe tube:

- 110 mm long, narrow, thick walled tube with 3mm internal bore.
- Graduated from 0-10 cm with graduation both in ascending and descending order on 2 sides of tube.
- The scale with the markings from 0-10 from above downwards is used in ESR determination and from below upward is used for Hematocrit (PCV) determination.



Procedure :

1. Under aseptic condition get a **venous blood sample**.
2. Carefully mix the blood sample in **EDTA vial** by repeated inversion.
3. Fill the Wintrobe tube with the help of **Pasteur pipette to the 10 cm** mark (which represent 100%).
4. Place the Wintrobe tube in the **centrifuge machine**, and other wintrobe tube filled **with water** on the opposite side **so as to balance** it.

PROCEDURE

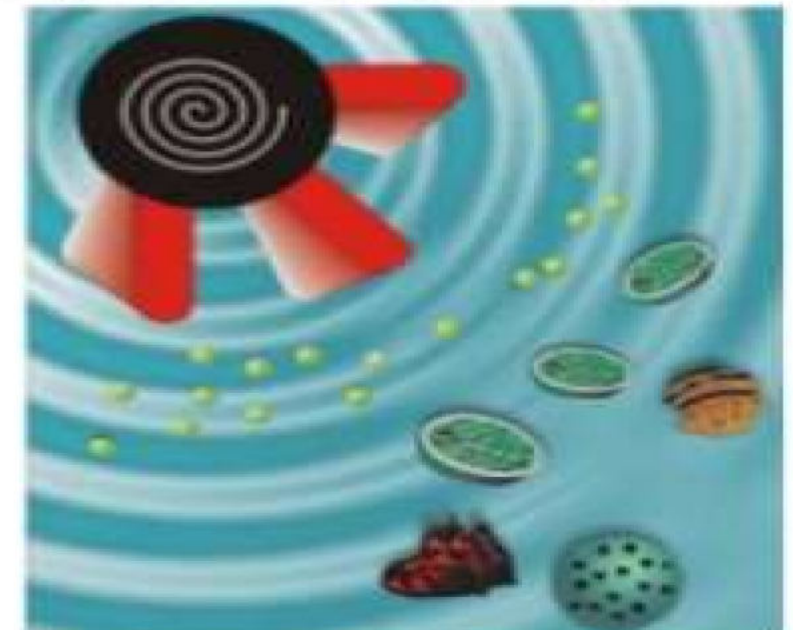
- Under aseptic condition get a venous blood sample.
- Carefully mix the blood with anticoagulant (Ammonium oxalate 3mg and Potassium Oxalate 2mg) in a tube by repeated inversions.
- Fill the Wintrobe tube with the help of Pasteur pipette to the 10cm mark. Which represents 100%.
- Place the Wintrobe tube in the centrifuge machine and other Wintrobe tube filled with water on the opposite side so as to balance it.

- Centrifuge for **30 minutes at 3000 rpm**.
- After 30 minutes stop the centrifuge machine.
- Take out the Wintrobe tube and **note the readings** directly off the graduation.



Principles of centrifugation

- A centrifuge is a device for separating particles from a solution according to their size, shape, density, viscosity of the medium and rotor speed.
- In a solution, particles whose density is higher than that of the solvent **sink (sediment)**, and particles that are lighter than it **float to the top**.
- The greater the difference in density, the faster they move. If there is no difference in density (isopyknic conditions), the particles stay steady.
- To take advantage of even tiny differences in density to separate various particles in a solution, gravity can be replaced with the much more powerful “**centrifugal force**” provided by a centrifuge.



OBSERVATION



- **Hematocrit**

- **Males: 44% (38—50%)**
- **Females: 42% (36—45%)**

Precautions

- There should be no air bubble or froth of blood in the tube.
- Always use double oxalate as anticoagulant.
- Wintrobe tube should be clean and dry.

DOUBLE OXALATE

- Acts by *chelating calcium in blood*
 - Also k/a Ammonium & Potassium oxalate
OR Heller and Paul double oxalate
 - Ammonium salt : causes swelling of RBC
 - Potassium salt : causes cell shrinkage
- hence double oxalate retains normal shape of RBC
proportion of potassium : ammonium :: 2:3

Sources of error

1. Improper sealing of the capillary tube.
2. An increased amount of anti-coagulant .
3. The time and speed of the centrifugation and the time when the results are read are very important.

4. If too much time elapses between when the centrifuge stops and the capillary tube is removed.
5. The buffy coat of the specimen should not be included in the Hct reading.
6. A decrease or increase in the readings may be seen if the microhematocrit reader is not used properly.

Hematocrit reader

➤ Reference ranges:

- | | |
|----------------|--------|
| • Newborn | 53-65% |
| • Infant/child | 30-43% |
| • Adult male | 42-52% |
| • Adult female | 37-47% |

True hematocrit

- After centrifugation small amount of plasma is still entrapped between RBCs. So the true Hematocrit is calculated as:
- Hematocrit $\times \frac{96}{100} = \text{True Hematocrit}$

Buffy Layer

- The buffy layer consists of packed platelets and leukocytes
- Thickness 1mm
- The thickness of buffy layer increases in leucocytosis ,leukaemia and thrombocytosis

Clinical Importance

High Hematocrit Level

Some factors may cause a rise in hematocrit value such as:

- Polycythemia
- Heart or kidney problems
- Intake of anabolic steroids
- Dehydration
- Diarehea
- Lung problems
- Burns
- Smoking
- High altitudes

Low Hematocrit Levels

The most common symptoms of low hematocrit levels are: fatigue, weakness, dizziness. Low hemoglobin levels and pregnancy is commonly associated with a decreased hematocrit range.

Other causes are:

- Anemia
- Hemolysis
- Nutritional deficiencies
- Bleeding
- Renal failure
- Pregnancy
- Medications

Physiologic (Normal) increase in PCV

- New born and Infants
- High altitude
- Higher in man as compared to female

Physiologic (Normal) decrease in PCV

- Less in women as compared to man
- Pregnancy (hemodilution)